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Supplementary Information for

The Novel PII-Interacting Regulator PirC (Sll0944) Identifies 3-Phosphoglycerate Mutase (PGAM) as Central Control Point of Carbon Storage Metabolism in Cyanobacteria

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SI Material and Methods

Strains and cultivation

A list of all used strains for this study is provided in Table S1.

For preculturing and growth experiments, the *Synechocystis* sp. PCC 6803 (from now on *Synechocystis*) strains were cultivated in BG_{11} medium according to Rippka (1). The cultivations were performed in Erlenmeyer flasks without baffles whereby 50 ml cultures were cultivated in 200 ml flask and 200 ml cultures in 500 ml flasks. Typical cultivation was performed either at continuous illumination (~50 µE m^{-2} s^{-1}) or light-dark conditions (12 h light and 12 h darkness) and 28 °C while the cultures were shacked continuously at 125 rpm. For nitrogen depletion, cultures were cultivated in BG11 media without 17,65 mM $NaNO_3$. Whenever necessary, appropriate antibiotics were added to the different strains to ensure the continuity of the mutation.

For nitrogen deficiency experiments, pre-cultures of *Synechocystis* were cultivated for three days as described previous at an initial OD_{750} of 0.1. Experimental cultures were then prepared in BG11 medium with a set starting OD_{750} of 0.2 and grown for two days under identical conditions until they reached an OD_{750} of 0.6-0.8. For the nitrogen shift experiments, cells from the cultures were harvested by centrifugation (4000 g, 10 min), washed with and resuspended in $BG11_0$ medium to create cultures with an initial OD_{750} of 0.4.

Cultivation of *Escherichia coli* cultures was performed with LB medium and agar. Lennox broth: 5 $g \cdot l^{-1}$ Yeast extract, 10 $g \cdot l^{-1}$ Tryptone, NaCl 5 $g \cdot l^{-1}$, solid: 15 $g \cdot l^{-1}$ agar

Plasmids and cloning

A list of all used primer, plasmids and its cloning procedure are listed in Table S2 and Table S3 as well as in Figure S4.

Overexpression and Purification of Proteins

Escherichia coli Lemo21(DE3) were used for overexpression of the various kind of proteins. The expression of His-tagged proteins was performed as described in the manufactured expression protocol in 2-fold concentrated LB media. An overnight expression, induced by addition of 400 µM IPTG, in a 1 l culture was performed with 1 mM L-rhamnose at 25 °C during continuous shaking at 120 rpm. Additionally, dependent on the plasmid the appropriated amount of the antibiotic was added to the culture. The expression of Strep-tagged proteins based on pASK-Iba5Plus expression plasmid was induced by addition of 200 $\mu g \cdot l^{-1}$ anhydrotetracycline without addition of L-rhamnose because of the T7 RNA polymerase independent expression.

The heterologous proteins containing His-tags were purified via 1 ml Ni-NTA HisTrap columns (GE Healthcare). The cells were lysed in 50 ml lysis buffer containing 50 mM Na-phosphate buffer pH 8, 300 ml NaCl, 1 mM DTT, 1 mM Benzamidine and 0,2 mM PMSF. The His-tagged proteins were loaded on the Ni-NTA column with Buffer A containing 50 mM Na-phosphate pH 8, 300 ml NaCl and eluted via a gradient of increasing imidazole (0-500 mM, Buffer B) using a ÄKTAPurifier™ System (GE Healthcare). After this first purification, the proteins were further purified via size exclusion chromatography using a Superdex™ 200 Increase 10/300 GL (GE Healthcare) with 50 mM Tris/HCl buffer containing 100 mM KCl and 0.5 mM EDTA.

For purification of Strep-tagged proteins, 5 ml Strep-tactin® superflow columns were used. Cells were lysed in lysis buffer containing 100 mM Tris/HCl pH 8, 150 mM NaCl, 1 mM EDTA and 1mM PMSF. The proteins were loaded on the column and eluted with buffer containing 5 mM Desthiobiotin. The buffer of each purified protein was exchanged via dialysis using dialysis buffer (50 mM Tris/HCl pH 7.8-8, 100 mm KCl, 5 mM $MgCl_2$, 0,5 mM EDTA, 40 % glycerol) and a 3 kDa cutoff dialysis tube. All purification steps were checked via SDS-PAGE according to previous studies (2).

The His-tag of PGAM was removed by Thrombin cleavage using Thrombin of bovine of Sigma Aldrich according to protocol (3).

In-batch Pulldown assay

Interactions between PirC and PII were first checked via an in-batch pulldown experiment. For this, His_6-PirC and Strep-PII were mixed in equimolar amounts and incubated for 20 min at room temperature in buffer containing 50 mM Tris/HCl, pH 7.8, 100 mM KCl, 0.5 mM EDTA, 10 mM $MgCl_2$ and additionally either 2 mM ATP, ADP or ATP and 2-OG. The mixtures were applied to Strep-Tactin XT coated magnetic beads. After 30 min of incubation the magnetic beads were removed via a strong magnet, followed by three times washing step with incubation buffer. For analysis of bound proteins via SDS-PAGE, the beads were boiled 10 min at 100 °C as described previously (2).

Co-Immunoprecipitation and Liquid chromatography-Mass spectrometry (LC-MS/MS)

To identify putative interaction partners of PirC, co-immunoprecipitation experiments were performed. For this, *Synechocystis* ΔPirC::PirC-mCitrine cultures were pre-cultivated in100 ml BG11 medium to an OD_{750} of ~0,8 and subsequently shifted to N-depleted medium. For Co-IP experiments in presence of the PII effector molecules, cells were harvested after 24 h N-depletion by centrifugation for 10 min at 4200 x g at 4 °C, followed by resuspension of the cell pellet in 2 ml binding buffer containing 100 mM TRIS (pH 7.5), 100 mM KCl, 1 mM $MgCl_2$, 1 mM DTT, 0.5 mM EDTA, 2 mM ATP and 2-OG. The cells were lysed with 150 µl glass beads in 1.5 ml screw cap tubes by harsh shaking in a high-speed homogenizer for 5 times 30 sec shaking at speed of 7 $m \cdot s^{-1}$ with each 5 min break. The lysate was than centrifuged at 25,000 x g for 5 min at 4 °C and a supernatant volume corresponding to a protein yield of approx. 3 mg was used for the immunoprecipitation. Therefore, GFP-Trap Magnetic Agarose beads or control beads without antibodies were used according to the manufactured protocol (both Chromotek, Planegg-Martinsried, Germany). The loaded magnetic beads were heated for 10 min at 95 °C in SDS loading buffer for the dissociation of purified proteins. Protein solutions were subjected to short SDS-PAGE runs, in which proteins were allowed to migrate for 1.5 cm into 12% Bis-Tris Gels (Invitrogen) and then stained with Coomassie blue. Protein containing gel regions were isolated and subjected to InGel digestion with trypsin as described elsewhere (4). Tree independent experiments, each including a PirC-mCitrine and a control CoIP were performed in total. Peptides were subjected to a clean-up step using StageTips (5) and subsequently analyzed by mass spectrometry. LC-MS/MS analysis was performed on a Q Exactive HF mass spectrometer (ThermoFisher, Germany), using linear, segmented 60 min nanoLC RP gradients as described elsewhere (16). All raw data was processed using MaxQuant software suite (version 1.6.5.0) at default settings. MS2 peak lists were searched against a target-decoy database of the *Synechocystis* sp. PCC 6803 proteome, including the sequence of PirC (Sll0944)-mCitrine. Label free quantification was used to calculate LFQ intensities for each CoIP sample. Data from all experiments was analyzed via the Perseus software (version 1.6.5.0). For the identification of significantly enriched proteins in PirC-mCitrine CoIPs, a *t*-test was performed with the following requirements: each protein had to be detected in at least two replicates and an FDR of 0.01 at S0 = 0.1 was set.

Biolayer interferometry using the Octet K2 system

In vitro binding studies were done by Bio-layer interferometry (BLI) using Octet K2 system (FortéBio). The experiments were performed in HEPES buffer (20 mM HEPES-KOH pH 8.0, 5 mM $MgCl_2$, 0.005 % NP-40, For protein interactions of His_8-PII–strep-PirC 150 mM KCl and for the His_6-PGAM–strep-PirC interaction 10 mM $MnCl_2$ was added to the buffer. In the first step PII-His_8 (400 nM, trimeric) or PGAM-His_6 (500 nM) were immobilized on Ni-NTA sensors (FortéBio) followed by a 60 sec baseline measurement. For the binding of PirC, the biosensors were dipped into the PirC solution for 180 sec (Association), with concentrations ranged between 9.375 nM – 1500 nM.

Dependent on the experiment different effector molecules were added to the binding buffer, ADP, ATP and 2-OG in PII binding studies, and 2PG as well as 3 PGA in PGAM binding assays. The assay was terminated by a 300 sec dissociation step. To prevent false positive results in each experimental set one measurement without any interaction partner was performed. The biosensors were regenerated after each use with 10 mM glycine (pH 1.7) and 10 mM $NiCl_2$ as proposed in manufacturers recommendations. The recorded curves of a set were preprocessed by aligning to the average of the baseline step and to the dissociation step. The response in equilibrium (R_{eq}) was calculated using the Data Analysis Software of the Octet System. The Concentration versus R_{eq} plots were made for each set of experiments which were then used to calculate the Dissociation constant K_D.

Glycogen measurement

The determination of the glycogen content of *Synechocystis* cultures was performed according to previous studies (6, 7). The glycogen was isolated from cell pellets of 2 ml culture. The glycogen was hydrolyzed to glucose with 4,4 U · μl^{-1} amyloglucosidase from *Aspergillus niger* (Sigma Aldrich) for 2 h at 60 °C. The resulting glucose concentration was measured via o-toluidine assay (8). The samples were boiled in 1:6 dilution with a 6 % o-toluidine reagent (in glacial acetic acid) for 10 min, then cooled on ice and measured at 635 nm. The concentration of samples was calculated using a calibration curve of defined quantity of glucose (0, 10 µg, 50 µg, 100 µg, 250 µg, and 500 µg).

PHB Quantification

Polyhydroxybutyrate was detected by high-performance liquid chromatography as described previously (9). Eleven ml of chlorotic cultures were centrifuged at 4200 x g for 10 min and the cell pellet was vacuum dried in pre-balanced 2 ml reaction tubes. To calculate the cell dry weight (CDW) the tube was cradled again. Then the pellet was boiled for 60 min in concentrated H_2SO_4 (18 mol · l^{-1}). Thereby, the cells were lysed and PHB converted to crotonic acid. Next, 110 µl of this solution was diluted 1:10 with 14 mM sulfuric acid solution and centrifuged 5 min at 25,000 × *g* followed by another 1:2 dilution. After another centrifugation step 300 µl of the clear supernatant was used for analytical HPLC. Reversed-phase HPLC was performed using the Chromatography system HP1090 M, equipped with a thermostated autosampler and diode-array-detector, HP Kayak XM 600 workstation. The crotonic acid was detected by measuring the absorbance at 210 nm. The crotonic acid concentration of the samples was calculated using a calibration curve of defined concentrations (0.5 mg·ml^{-1}, 0.25 mg·ml^{-1}, 0.125 mg·ml^{-1} and 0.0625 mg·ml^{-1})

PGAM enzymatic assay

The PGAM activity and the effect of PirC was determined by a coupled enzyme assay as described previous (10, 11). For that, 10 µg of purified PGAM was used in a 1 ml reaction. The reaction mixture containing 20 mM HEPES-KOH (pH 8,0), 100 mM KCl, 5 mM $MgSO_4$, 0.4 mM $MnCl_2$, 50 µg·ml^{-1}BSA, 1 mM DTT, 0.4 mM ADP, 0.2 mM NADH, 0.5 U enolase (Sigma Aldrich), 2 U Pyruvate kinase (Sigma Aldrich), 2 U Lactate dehydrogenase (Roche) and 10 µg PGAM was pre-warmed to 30 °C. The Assay was started by adding the 3-PGA solutions. The resulted decrease of NADH over time was recorded with Specord50 (Jena Analytics) at 340 nm. A blank assay without 3-PGA was also performed, no decrease was detectable.

Phase contrast and fluorescence microscopy

The visualization of PHB granules was done by phase contrast fluorescence microscopy using the Leica DM5500 B with the Leica CTR 5500 illuminator. The integrated camera Leica DFC 360 FX was used for image acquisition. The settings were adjusted by the Leica Application Suite Advanced Fluorescence (LAS 4.0). The specimen was prepared by dropping 10 µl of cell culture on an agarose coated microscope slide and observed using the Leica HCX PL FLUATAR (100 x

1,30 PH3) with immersion oil for a 1000-fold magnification. The visual detection of PHB via fluorescence was performed by staining the cells with 0,33 $\mu g \cdot ml^{-1}$ Nile Red. The fluorescence of Nile Red was excited with light between 542 – 568 nm (CYR3 channel) and of GFP like proteins with light between 455-495 nm (GFP channel). The images were processed by 3D deconvolution of all channels using LAS.

Transmission electron microscopy

For electron microscopic pictures, *Synechocystis* cells were fixed with glutaraldehyde and post-fixed with potassium permanganate, respectively. Afterwards, microtome sections were stained with lead citrate and uranyl acetate (12). The samples were then examined using a Philips Tecnai 10 electron microscope at 80 kHz.

Metabolome analysis

For metabolome analysis by LC-MS *Synechocystis* was cultivated in 200 ml under N-depletion as described previously for 48 h under continuous lightning. The sampling was carried out 0, 6, 24 and 48 hours after the shift. Samples of 5 mL liquid culture were quickly harvested onto nitrocellulose membrane filters (ø 25 mm, 0.45 µm pore size, Porafil NC, Macherey-Nagel) by vacuum filtration, put in 2 ml Eppendorf microtubes, and immediately frozen in liquid nitrogen. Cells on filters were stored at -80 °C until analysis.

Extraction was done using LC-MS grade chemicals. To every filter, 630 µL methanol and 1 µL carnitine as internal standard (1 $mg \cdot ml^{-1}$) were added. Cells were re-suspended by rough mixing and then incubating samples in a sonication bath for 10 min. Samples were shaken for 15 min prior to addition of 400 µL chloroform and incubation at 37 °C for 10 min. Next, 800 µL of ultrapure water were added. The extracts were shaken for 15 min and then incubated at -20 °C for at least 2 h. Cell debris and filters were removed by centrifugation (20000 g, 5 min, 4 °C). The upper polar phase was transferred completely into a new microtube and subsequently dried by vacuum concentration (Concentrator plus, Eppendorf). The dried extracts were re-suspended in 200 µL deionized water and filtrated (0.2 µm filters, Omnifix-F, Braun). The filtrates were then analysed via the high-performance liquid chromatograph mass spectrometer LCMS-8050 system (Shimadzu), as previously described by Selim et al., 2018 (13). LC-MS data analysis was done using the Lab solution software package (Shimadzu).

Heading

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SI Figures

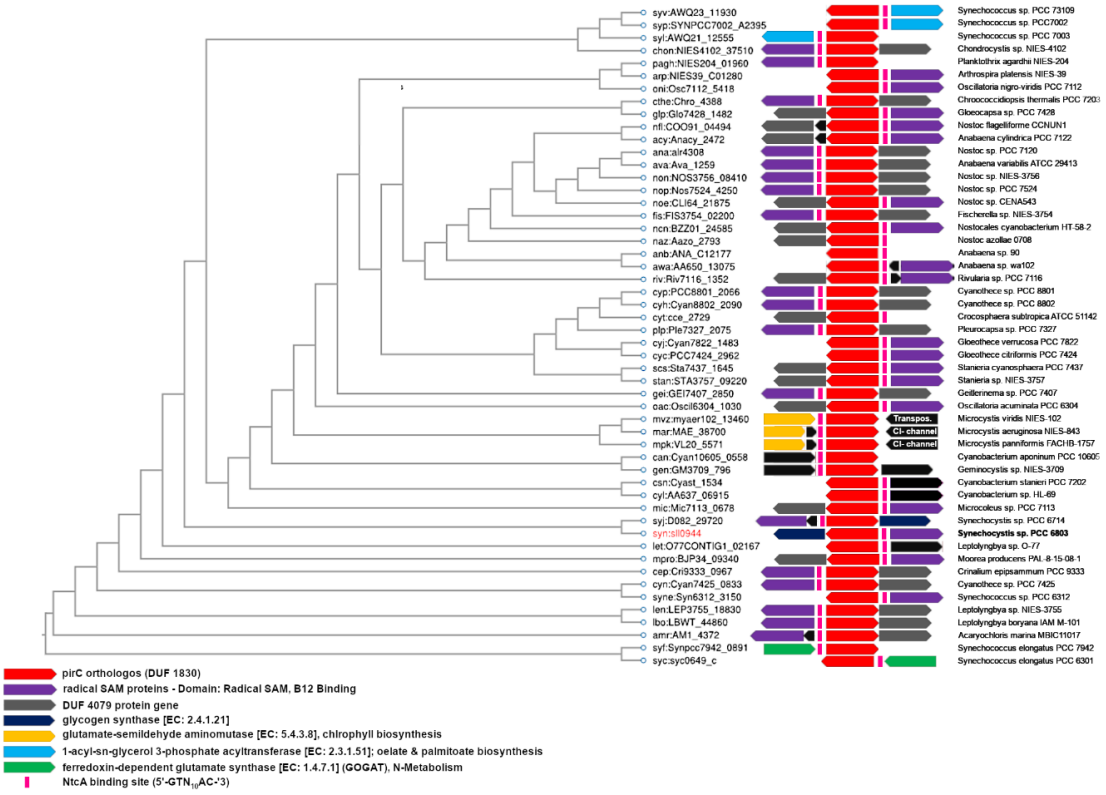

Fig. S1 - Cluster comparison of 53 different Cyanobacterial PirC orthologous. Detected by a Smith-Waterman (SW) algorithm based SSEARCH for bidirectional best hits of the KEGG SSDB (Sequence Similarity Database) with a SW-Score threshold of 100. Red arrows represents the pirC orthologous with direction. In front of each pirC gene a predicted NtcA binding site is present (5'-GTN10AC-'3, pink bars). In 67 % of the cases the pirC are next to genes encoding radical SAM-like proteins (purple arrows).

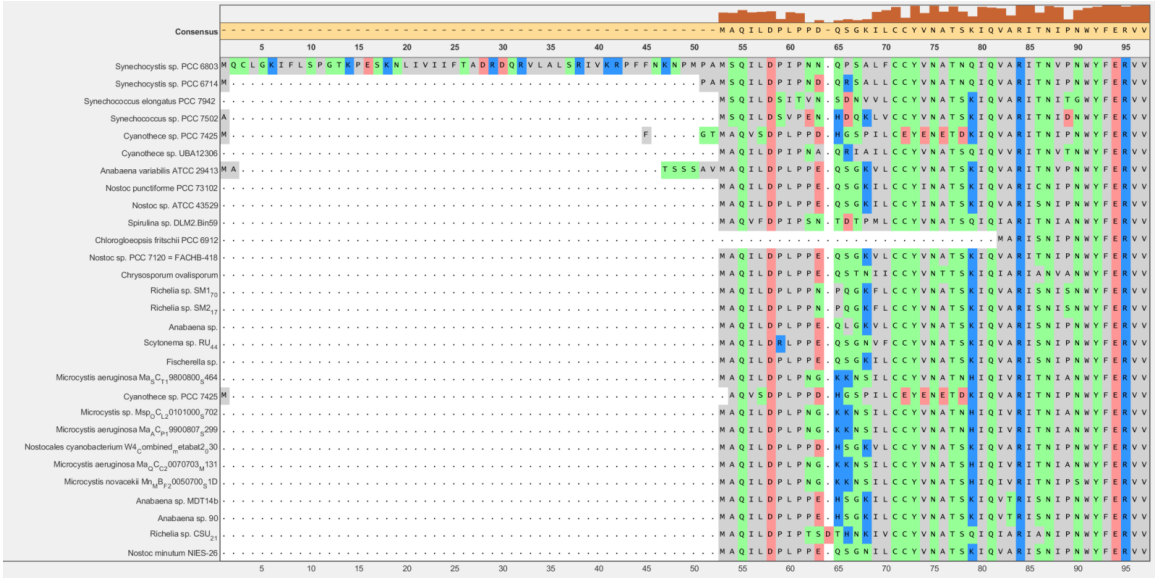

Fig. S2 – Multiple alignment of PirC orthologs of different cyanobacteria. It is shown that the first 52 AA are only present in *Synechocystis* sp. PCC 6803 PirC (first row). The picture shows extract of alignment of 29 different cyanobacterial orthologs of a multiple alignment of 74 different orthologs.

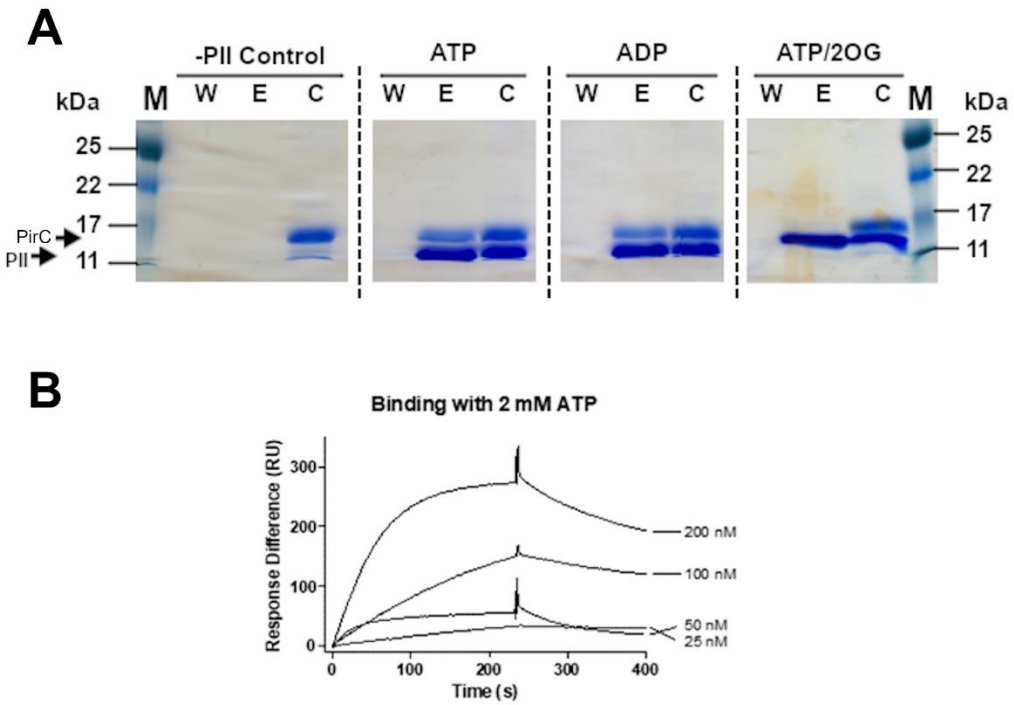

Fig. S3 - SDS-PAGE of in-batch pulldown assays in presence of 10 mM $MgCl_2$ and either 2 mM ATP, 2 mM ADP, or 2 mM ATP/2-OG**.** The first column represents the negative control without P_{II}**.** PirC was co-eluted with P_{II} attached to strep-tactin XT coated magnetic beads in presence of ATP and ADP, observable by to bands. However, there is no band in presence of ATP/2-OG. In the controls without P_{II}, no elution of PirC was observed. 1: Marker; W: washing fractions; E: elution fractions; C: control (reaction mix before the pulldown was performed).

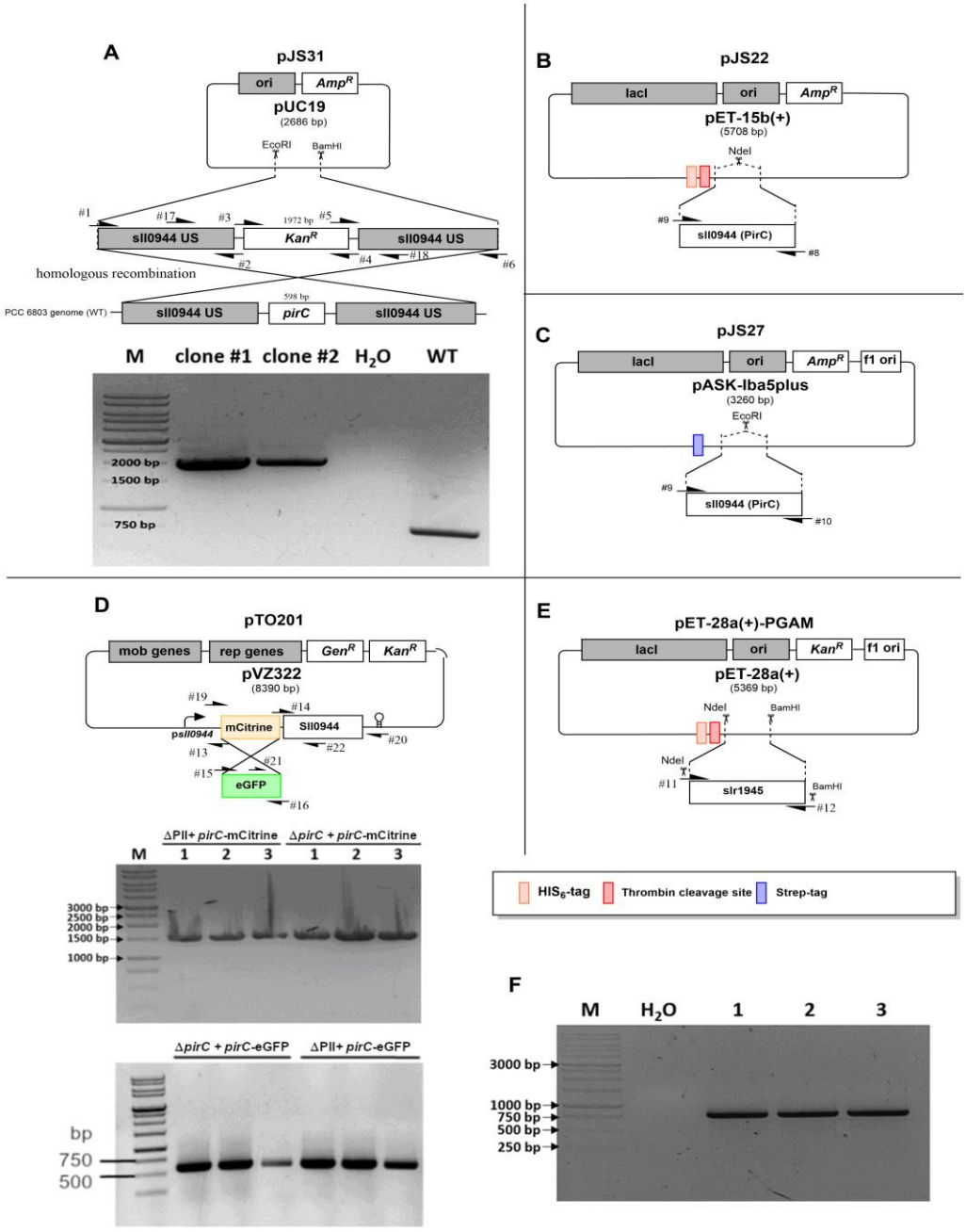

Fig. S4 - Plasmid Construction and mutant check in *Synechocystis* sp. PCC 6803. (A) Plasmid construction, gene deletion technique, manufactured by Gibson Assembly and colony PCR check of *Synechocystis* Δ*pirC* gene using primer pair #17/18 **(B)** Plasmid construction pJS22 for His_6-PirC expression in *E. coli* , manufactured by Gibson Assembly **(C)** Plasmid construction pJS27 for Strep-PirC expression in *E. coli* , manufactured by Gibson Assembly **(D)** Plasmid construction pTO201 for eGFP-PirC expression in *Synechocystis*, manufactured by Gibson Assembly as well as colony PCR of successful transformation of pVZ322-mCitrine-*pirC*-comp and pTO201.**(E)** Plasmid construction pET-28a(+)-PGAM for His6-PirC expression in *E. coli.* Manufactured by Restriction/ligation method **(F)** colony PCR of successful transformation of pVZ322-pirC-comp.

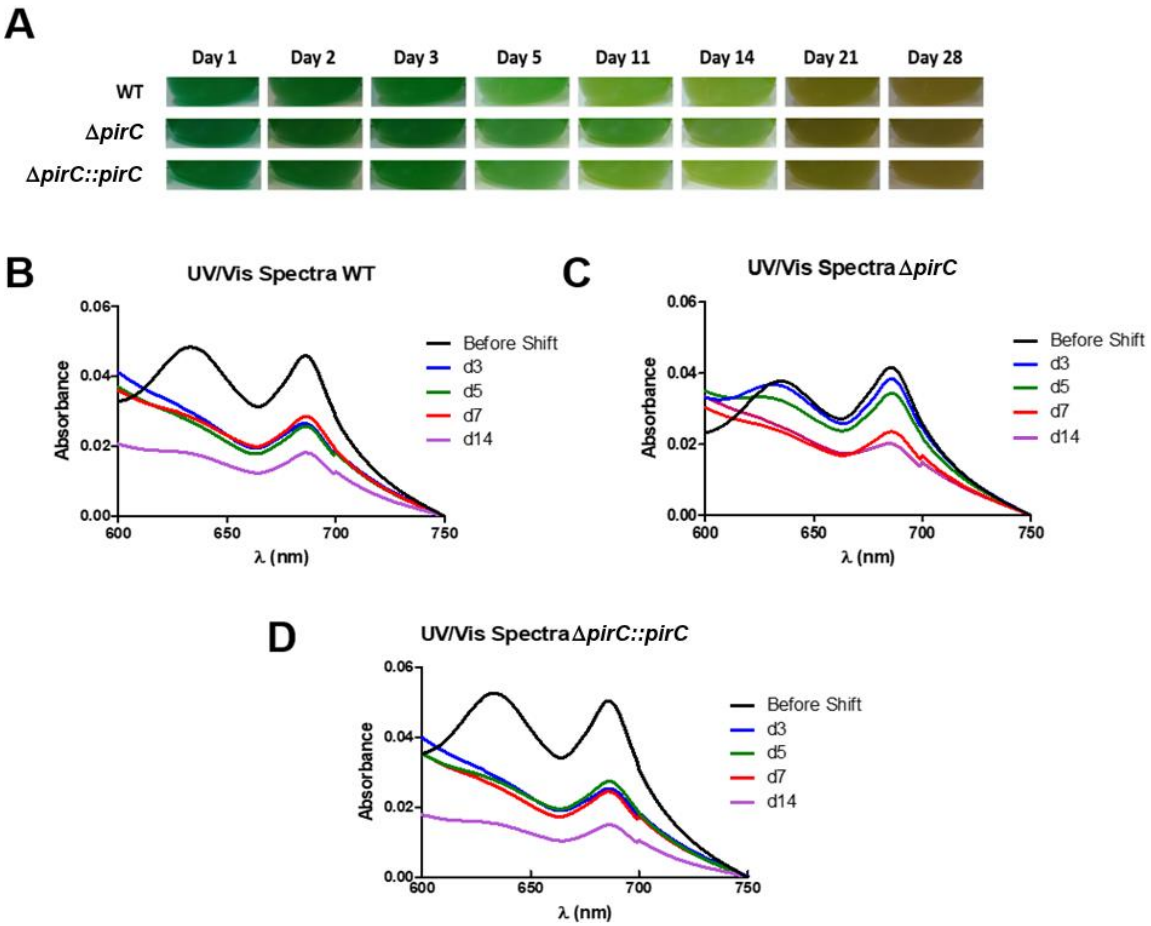

Fig. S5 - Chlorosis due to nitrogen depletion of *Synechocystis* wild type, Δ*pirC*, and Δ*pirC::pirC* in the day/night cabinet. (A) Photographs showing depigmentation of representative cultures from the three replicates; **(B)** UV/Vis spectra (mean of three replicates) of the wild type; **(C)** UV/Vis spectra (mean of three replicates) of the Δ*pirC* mutant; **(D)** UV/Vis spectra (mean of three replicates) of the complemented mutant Δ*pirC::pirC*.

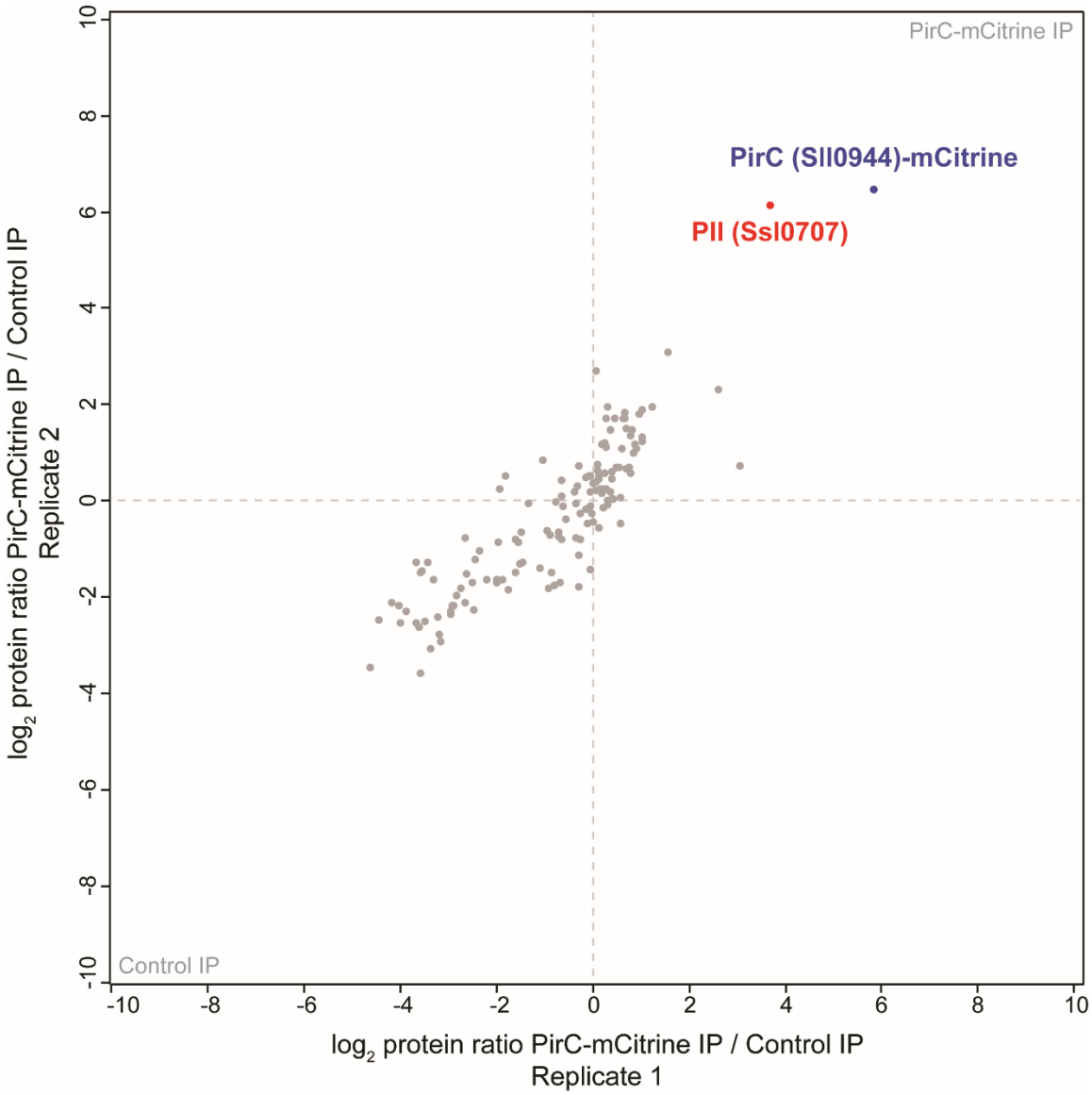

Fig. S6 - Validation of the interaction of the PirC-mCitrine construct with PII. The newly designed PirC-mCitrine fusion protein was tested in anti-GFP immunoprecipitation (IP) experiments for interaction with the PII protein at conditions of low ATP and 2-OG levels, as revealed in reverse IP experiments against a tagged PII construct (14). The scatterplot of two independent PirC-mCitrine IP experiments confirms a significant co-enrichment (p-value=0.01) of PirC and PII, indicated in blue and red, respectively. The IP was performed with crude cell extracts from nitrogen-starved cells of the Δ*pirC*::*pirC-mCitrine* strain, without addition of key metabolites. Extracts were either incubated with GFP-Trap coated magnetic agarose beads (PirC-mCitrine IP) or protein A/G agarose beads coated with an unrelated antibody (Control IP). IP eluates were differentially labeled by dimethylation labeling and analyzed by high accuracy LC-MS/MS. MS data was processed and analyzed as described elsewhere (14, 15).

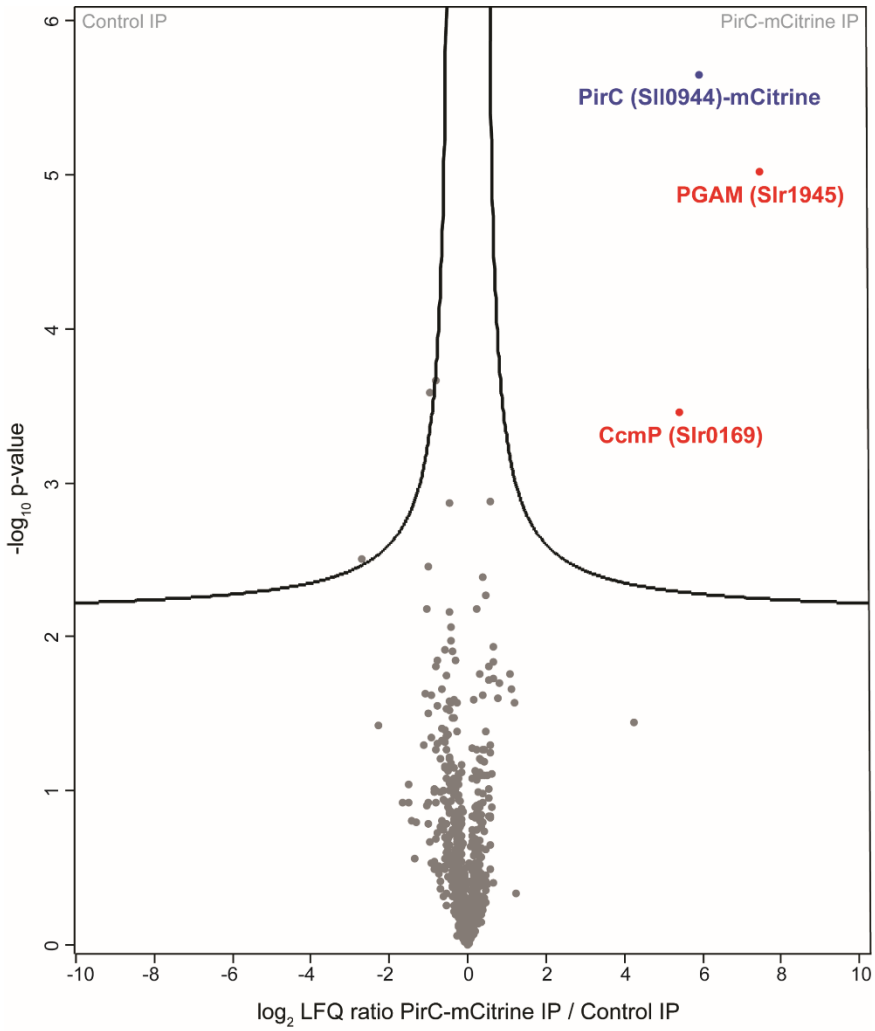

Fig. S7 - The PirC interactome at nitrogen starvation conditions. Volcanoplot of three independent PirC-mCitrine immunoprecipitation (IP) experiments displays co-enriched proteins. Crude cell extracts from the nitrogen-starved Δ*pirC*::*pirC-mCitrine* strain were incubated with either GFP-Trap coated magnetic agarose beads (PirC-mCitrine IP) or non-coated beads (Control IP) in presence of ATP, 2-OG and Mg^{2+}(each 2 mM). IP eluates were analyzed by high accuracy LC-MS/MS using label-free quantification to calculate protein enrichment ratios. Significantly enriched proteins were defined by *t*-test (FDR=0.01; S0=0.1) and are indicated in blue (PirC-mCitrine) or red (co-enriched proteins).

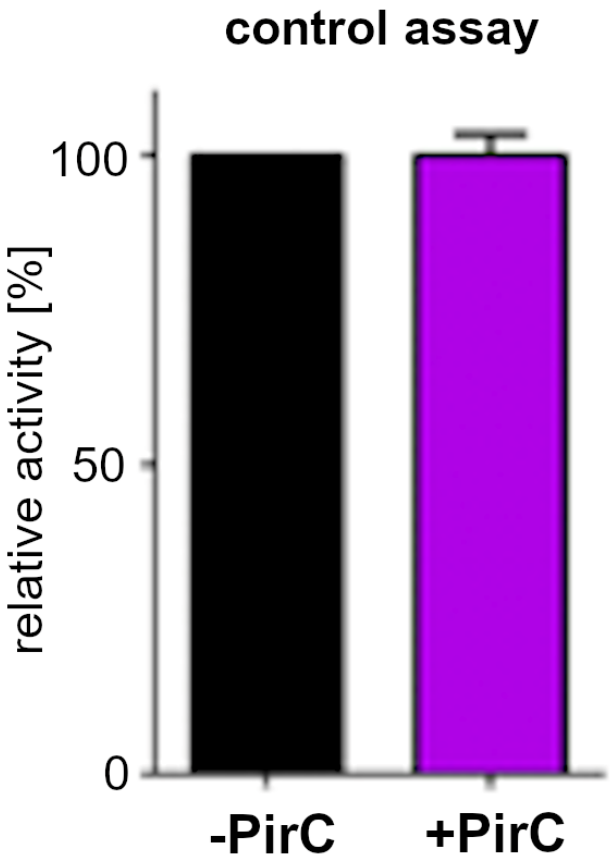

Fig. S8 – Control assay of coupled enzymes. Black bar represents the mean of triplicates with SD of control assay without PirC addition. Purple bar represents the mean of triplicates with SD of control assay with the addition of 600 nM PirC. In the assay 0.625 mM 2-PGA was added to the assay.

Fig. S9 – LC-MS analysis of the hole spectrum of measured compounds. On x-axis the concentration of the compounds is shown in ng · OD_{750}^{-1} · ml^{-1}**.** Each bar represents the mean of two technical replicates of two biological replicates. The Error bar represents the SD of the measurement.

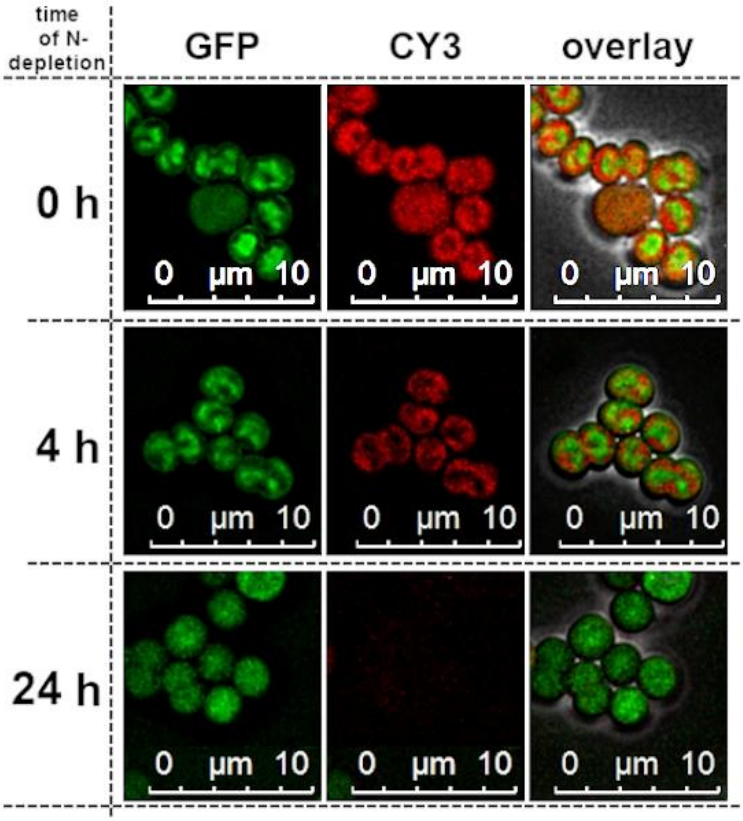

Figure S10 - Intracellular localization of the PirC-eGFP fusion protein in *Synechocystis* sp. PCC 6803. Change of the intracellular localization of the PirC-eGFP signal in *Synechocystis* after nitrogen depletion. Representative pictures of three biological replicates. Directly after the shift to nitrogen depleted conditions, the signal is localized centrally in the cytoplasm (0h) with no clear change after 4 h (4 h). However, the signal is more distributed throughout the cell after 24 h.

Table S1 - Used Organisms and strains in this study

Organism	**Strain**	**Genotype**	**Purpose**
E. coli	Top10	F- mcrA Δ(mrr-hsdRMS-mcrBC) Φ80lacZΔM15 ΔlacX74 recA1 araD139 Δ(araleu)7697 galU galK rpsL (StrR) endA1 nupG	Molecular Cloning
E. coli	NEB10β	Δ(ara-leu) 7697 araD139 fhuA ΔlacX74 galK16 galE15 e14- Φ80dlacZΔM15 recA1 relA1 endA1 nupG rpsL (StrR) rph spoT1 Δ(mrr-hsdRMS-mcrBC)	Molecular Cloning
E. coli	Lemo21(DE3)	fhuA2 [lon] ompT gal (λ DE3) [dcm] ΔhsdS/ pLemo(CamR) λ DE3 = λ sBamHIo ΔEcoRI-B int::(lacI::PlacUV5::T7 gene1) i21 Δnin5 pLemo = pACYC184-PrhaBAD-lysY	Protein Expression
E. coli	Lemo21(DE3) + pJS15	Lemo21(DE3) + pJS15	Expression of strep-Tagged PII protein
E. coli	Lemo21(DE3) + pJS22	Lemo21(DE3) + pJS22	Expression of His_8-Tagged PirC
E. coli	Lemo21(DE3) + pJS26	Lemo21(DE3) + pJS26	Expression of His_8-Tagged PirC
E. coli	Lemo21(DE3) + pJS27	Lemo21(DE3) + pJS27	Expression of strep-Tagged PirC
E. coli	Lemo21(DE3) + pET-28a(+)-PGAM	Lemo21(DE3) + pET-28a(+)-PGAM	Expression of His_6-Tagged PGAM
Synechocystis sp. PCC 6803	wild type glucose sensitive	WT	Background strain, control
Synechocystis sp. PCC 6803	ΔPII	ssl0707::$Spec^R$	Control strain
Synechocystis sp. PCC 6803	Δ*pirC*	*pirC*::Kan^R	Characterization of sll0944 KO mutants
Synechocystis sp. PCC 6803	Δ*pirC*::*pirC*	*pirC*::Kan^R pVZ322-pirC	Complementation of sll0944 knock out
Synechocystis sp. PCC 6803	Δ*pirC*::*pirC*-mCitrine	Δ*pir*C pVZ322-pirC-mCitrine	Localization of PirC in Δ*pirC* and Co-immunoprecipitation
Synechocystis sp. PCC 6803	Δ*pirC*::*pirC*-eGFP	Δ*pir*C pVZ322-*pirC*- eGFP	Localization of PirC in Δ*pirC* and Co-immunoprecipitation

Table S2 - Used Primer in this study

#No	Primer	Sequence (5'-3')
#1	pUC19-pirCUS_fw	GTTTTCCCAGTCAGACGTTGTAAAACGACGGCCAGTGAATTGTCGGCTGAAATTCATC
#2	pirCUS-KanR_rev	GAATTGACATAAGCCTGTTCAATCAAATTTTTTGACTCTG
#3	pirCUS-KanR_fw	CAGAGTCAAAAAATTTGATTCAACAGGCTTATGTCAATTC
#4	KanR-pirCDS_rev	CCAATTCATTAATTCATTGGAGTTTGTAGAAACGCAAAAA
#5	KanR-pirCDS_fw	GCCATCCTGACGGATGGCCTTTTTGCGTTTCTACAAACTCCAATGAATTAATGAATTGG
#6	pirCDS-pUC19_rev	CGCCAAGCTTGCATGCCTGCAGGTCGACTCTAGAGGATCTGGAACATGGCTTCCCCTTTC
#7	pET15b-pirC(correct)_fw	CATCATCATCACAGCAGCGGCCTGGTGCCGCGCGGCAGCATGTCGCAAATCTTGGACCC
#8	pirC-pET15b_rev	CCTTTCGGGCTTTGTTAGCAGCCGGATCCTCGAGCATACTATGCGACAAGAGATTGAC
#9	pASKIba5Plus-pirC_fw	CCACCCGCAGTTCGAAAAAGGCGCCGAGACCGCGGTCCCGATGTCGCAAATCTTGGACCC
#10	pASKIba5Plus-pirC_fw	GGTCGACCTCGAGGGATCCCCGGGTACCGAGCTCGAATTCTATGCGACAAGAGATTGAC
#11	Slr1945-NdeI-fw	CATATGATGGCAGAGGCACCGATCGCC
#12	Slr1945-BamHI-rv	GGATCCCTAACGGGAGAGATTGACCGG
#13	pVZmCit_pirC_fwd	CTGCAGGAGCAGAAGAGCATAC
#14	pVZmCit_ pirC_rev	AGCCACTAAGGATTGGGAAG
#15	pTO201_eGfp_fwd	ACACGAGTCCGAGGATATGACTTCCCAATCCTTAGTGGCTATGAGTAAAGGAGAAGAAC
#16	pTO201_eGFP_rev	CTGGCTTTGCTTCCAGATGTATGCTCTTCTGCTCCTGCAGTTATTTGTATAGTTCATCCATGC
#17	6803 pirCKOcheck_fw	TGGCATGGCCTAAGTATTCC
#18	6803 pirCKOcheck_rev	GCGTTCTGCAGGGGATTACC
#19	pVZ322seq_fw	CCTGGCTTTGCTTCCAGATG
#20	pVZ322seq_rev	TGCCCGGATTACAGATCCTC
#21	seq_pTO201_fwd	CAATGCTTTGCGAGATACCC
#22	seq_pTO201_rev	AGCTCCATAGGCCGCTTTC

Table S3 – Plasmids used in this study

Plasmid	Purpose	Source
pJS15	Expression of Strep-Tagged PII protein (Ssl0707) in E. coli	(16)
pJS22	Expression of His_8-Tagged PirC (Sll0944) in E. coli T7-strains	This study
pJS26	Expression of His_8-Tagged PII protein (Ssl0707) in E. coli T7-strains	(16)
pJS27	Expression of strep-Tagged PirC (Sll0944) in E. coli	This study
pJS31	Kan^R deletion of the pirC gene in Synechocystis sp. PCC 6803	This study
pET-28a(+)-PGAM	Expression His_8-Tagged PGAM (Slr1945) in E. coli T7-strains	This study
pVZ322-pirC-comp	Complementation of pirC deletion	(17)
pVZ322-mCitrine-pirC-comp	Complementation of pirC deletion tagged to mCitrine protein	(17)
pTO201	Complementation of pirC deletion tagged to eGFP protein	This study

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